

CLAIMS

What we claim is:

1. A nucleic acid molecule comprising a promoter functional in *E. coli* and operatively coupled to a modified operon of a non-typeable strain of *Haemophilus* comprising A, B and C genes, wherein the A gene of the operon contains only a nucleic acid sequence which codes for a mature high molecular weight protein of the non-typeable strain of *Haemophilus*.
2. The nucleic acid molecule of claim 1 wherein said promoter is the T7 promoter.
3. The nucleic acid molecule of claim 1 wherein said operon encodes the high molecular weight protein (HMW1) of the non-typeable strain of *Haemophilus*.
4. The nucleic acid molecule of claim 1 wherein said a non-typeable strain of *Haemophilus* is selected from the group consisting of strains 12, Joyc, K21, PMH1 and 15 of non-typeable *Haemophilus influenzae*.
5. The nucleic acid molecule of claim 1 wherein said operon encodes the high molecular weight protein 2 (HMW2) of the non-typeable of *Haemophilus*.
6. The nucleic acid molecule of claim 5 wherein the non-typeable strain of *Haemophilus* is selected from the group consisting of strains 12, Joyc, K21, LCDC2, PMH1 and 15 of non-typeable *Haemophilus influenzae*.
7. The nucleic acid molecule of claim 1 wherein said nucleic acid sequence which codes for a mature high molecular weight protein has a nucleic acid sequence selected from those having SQ ID NOS: 27, 31, 36, 40, 44, 48, 52, 56, 60, 64, 68, 72.
8. The nucleic acid molecule of claim 1 wherein said nucleic acid sequence which codes for a mature high molecular weight protein encodes a HMW1 or HMW2 protein

having an amino acid sequence selected from those having SEQ ID NOS: 28, 32, 37, 41, 45, 49, 53, 57, 61, 65, 69, 73.

9. The nucleic acid molecule of claim 1 further comprising an additional nucleic acid sequence encoding the mature high molecular weight protein of a non-typeable strain of *Haemophilus*.

10. The nucleic acid molecule of claim 1 further comprising the *cer* gene of *E. coli*.

11. The nucleic acid molecule of claim 1 further comprising the *cer* gene of *E. coli* and an additional nucleic acid sequence encoding the mature high molecular weight protein of a non-typeable strain of *Haemophilus*.

12. An isolated and purified nucleic acid molecule encoding a high molecular weight (HMW) protein of a non-typeable strain of *Haemophilus influenzae* having:

(a) a DNA sequence selected from the group consisting of those shown in Figures 18, 19, 20, 21, 22, 23, 24, 25, 26 and 27 (SEQ ID NOS: 25, 27, 29, 31, 33, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64); or

(b) a DNA sequence encoding a high molecular weight protein having an amino acid sequence selected from the group consisting of those shown in Figures 18, 19, 20, 21, 22, 23, 24, 25, 26 and 27 (SEQ ID NOS: 26, 28, 30, 32, 34, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65).

13. A vector adapted for transformation of a host comprising the nucleic acid molecule of claim 1.

14. The vector of claim 13 which is a plasmid vector.

15. The vector of claim 14 wherein said plasmid has the identifying characteristic of a plasmid which is selected from group consisting of :

| | |
|-------------|--------------------|
| DS-1046-1-1 | (ATCC No.: 203263) |
| JB-2507-7 | (ATCC No.: 203262) |
| BK-86-1-1 | (ATCC No.: 203258) |
| BK-35-4 | (ATCC No.: 203259) |
| BK-76-1-1 | (ATCC No.: 203261) |
| DS-2334-5 | (ATCC No.: 203260) |
| DS-2400-13 | (ATCC No.: 203257) |

16. A strain of *E. coli* transformed by an expression vector of claim 14 and expressing a protective high molecular weight protein of a non-typeable strain of *Haemophilus*.

17. A recombinant protective high molecular weight protein of a non-typeable strain of *Haemophilus* or immunogenic fragment or analog thereof producible by the transformed *E. coli* strain of claim 16.

18. A plasmid vector for expression of a high molecular weight protein of a non-typeable strain of *Haemophilus* and comprising the T7 promoter, a cloning site for insertion of a nucleic acid molecule into the plasmid vector and the portions B and C of the operon of a non-typeable *Haemophilus* strain.

19. The plasmid of claim 18 further comprising the *E. coli* *cer* gene.

20. The plasmid vector of claim 18 which has the identifying characteristics of a plasmid which is plasmid JB-2646-1 (ATCC NO. 203256).

21. An isolated and purified protective HMW 1 protein of a strain of non-typeable *Haemophilus* which is free from contamination by the HMW2 protein of the same strain of non-typeable *Haemophilus*.

22. An isolated and purified protective HMW2 protein of a strain of non-typeable *Haemophilus* which is free from contamination by the HMW1 protein of the same strain of non-typeable *Haemophilus*.

23. The HMW1 or HMW2 protein of claim 21 or 22 wherein said non-typeable strain of *Haemophilus* is selected from the group consisting of strains Joyc, K21, LCDC2, PMH1 and 15 of non-typeable *Haemophilus influenzae*.

24. The HMW1 or HMW2 protein of claim 21 or 22 which has an amino acid sequence selected from the group consisting of SEQ ID NOS: 28, 32, 37, 41, 45, 49, 53, 57, 61, 65, 69, 73.

25. An immunogenic composition, comprising at least one immunogenically-active component selected from the group consisting of:

(A) a nucleic acid molecule comprising a promoter functional in *E. coli* and operatively coupled to a modified operon of a non-typeable strain of *Haemophilus* comprising A, B and C genes, wherein the A gene of the operon contains only a nucleic acid sequence which codes for a mature high molecular weight protein of the non-typeable strain of *Haemophilus*;

(B) an isolated and purified nucleic acid molecule encoding a high molecular weight (HMW) protein of a non-typeable strain of *Haemophilus influenzae* having:

(a) a DNA sequence selected from the group consisting of those shown in Figures 18, 19, 20, 21, 22, 23, 24, 25, 26 and 27 (SEQ ID NOS: 25, 27, 29, 31, 33, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64); or

(b) a DNA sequence encoding a high molecular weight protein having an amino acid sequence

selected from the group consisting of those shown in Figures 18, 19, 20, 21, 22, 23, 24, 25, 26 and 27 (SEQ ID NOS: 26, 28, 30, 32, 34, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65);

(C) a recombinant protective high molecular weight protein of a non-typeable strain of *Haemophilus* or an immunogenic fragment or analog thereof producible by a strain of *E. coli* transformed by an expression vector comprising a nucleic acid molecule comprising a promoter functional in *E. coli* and operatively coupled to a modified operon of a non-typeable strain of *Haemophilus* comprising A, B and C genes, wherein the A gene of the operon contains only a nucleic acid sequence which codes for a mature high molecular weight protein of the non-typeable strain of *Haemophilus*;

(D) an isolated and purified protective HMW 1 protein of a strain of non-typeable *Haemophilus* which is free from contamination by the HMW2 protein of the same strain of non-typeable *Haemophilus*;

(E) an isolated and purified protective HMW2 protein of a strain of non-typeable *Haemophilus* which is free from contamination by the HMW 1 protein of the same strain of non-typeable *Haemophilus*; and

a pharmaceutically acceptable carrier therefor.

26. The immunogenic composition of claim 25 formulated as a vaccine for in vivo administration to protect against diseases caused by *Haemophilus*.

27. The immunogenic composition of claim 25 formulated as a microparticle, capsule or liposome preparation.

28. The immunogenic composition of claim 25 in combination with a targeting molecule for delivery to

specific cells of the immune system or to mucosal surfaces.

29. The immunogenic composition of claim 25 further comprising an adjuvant.

30. A method for inducing protection against disease caused by *Haemophilus*, comprising administering to a susceptible host an effective amount of the immunogenic composition of claim 25.

31. The method of claim 30, wherein the susceptible host is a human.

32. A method of the production of a protective high molecular weight protein of a non-typeable strain of *Haemophilus*, which comprises:

transforming *E. coli* with a vector as claimed in claim 14,

growing *E. coli* to express the encoded mature high molecular weight (HMW) protein, and

isolating and purifying the expressed HMW protein.

33. The method of claim 32 wherein said non-typeable strain of *Haemophilus* is selected from the group consisting of strains 12, Joyc, K21, LCDC2, PMH1 and 15 of non-typeable *Haemophilus*.

34. The method of claim 32 wherein the high molecular weight protein is an HMW1 protein of the non-typeable strain of *Haemophilus*.

35. The method of claim 32 wherein the high molecular weight protein is an HMW2 protein of the non-typeable strain of *Haemophilus*.

36. The method of claim 32 wherein said isolation and purification procedure includes separating the HMWA protein from the B and C proteins.